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Lipase - Catalyzed glycerolysis of sunflower oil to produce partial glycerides

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RESUMEN

Glicerolisis de aceite de girasol catalizada por lipasa para producir glicéridos parciales.

Se prepararon glicéridos parciales mediante glicerolisis de aceite de girasol en presencia de lipasa como catalizador. Seis lipasas de orígenes diferentes se utilizaron y compararon en función de su actividad catalítica. Estas incluyeron lipasa de *Chromobacterium,* lipasa pancreática, lipasa de *Rhizopus arrhizus,* lipasa liofilizada (lipasa vegetal) además de dos preparaciones de lipasa derivadas de *Rhizopus japonicus:* lilipase A-10 y lilipase B-2.

Se encontró que la lipasa de *Chromobacterium* fue la más activa como catalizador en la glicerolisis mientras que la lipasa liofilizada, preparación vegetal a partir de germen de trigo, fue la menos activa.

Los resultados mostraron que los tipos de lipasa afectan también a la polaridad de los productos y por tanto a los rendimientos en su aplicación como emulsificantes alimentarios. Los productos menos polares pueden obtenerse usando lipasa de *Chromobacterium* mientras que los más polares se obtienen usando las preparaciones de lipasa de hongo «Lilipase A-10». La polaridad del producto está también influenciada por la temperatura del proceso aunque la forma de su efecto es distinta para las diferentes lipasas.

PALABRAS-CLAVE: Aceite de girasol — Glicérido parcial (obtención) — Glicerolisis — Lipasa.

SUMMARY

Lipase - Catalyzed glycerolysis of sunflower oil to produce partial glycerides.

Partial glycerides were prepared by glycerolysis of sunflower oil in presence of lipase enzyme as catalyst. Six lipases of different origins were used and compared for their catalytic activity. These include *Chromobacterium* lipase, pancreatic lipase, *Rhizopus arrhizus* lipase, lyophilized lipase (plant lipase) in addition to two lipase preparations derived from *Rhizopus japonicus;* Lilipase A-10 and Lilipase B-2. *Chromobacterium* lipase was found to be the most active as glycerolysis catalyst whereas lyophilized lipase; a plant preparation from wheat germ was the least active.

The results have also shown that the lipase type affects also the product polarity and hence its field of application as a food emulsifier. Less polar products can be obtained using *Chromobacterium* lipase whereas the more polar ones using a fungal lipase preparation «Lipase A-10». The product polarity is also influenced by the process temperature but the mode of its effect is different for different lipases.

KEY-WORD: Glycerolisis — Lipase — Partial glyceride (obtention) — Sunflower oil.

1. INTRODUCTION

Partial glycerides; mono and diglycerides constitute the major type of food emulsifers used in many food systems and are also important as a basic starting material to prepare several other derivatives of modified functional properties. Partial glycerides can be prepared by two processes; direct esterification of glycerol with fatty acids or índirect esterification of glycerol with oils or fats (glycerolysis). Each of the two processes involves several consecutive and reversible reaction steps. Several factors can markedly affect the rate of each step and consequently the yield and composition of the net product. Therefore, a successful industrial production of partial glycerides of a certain desirable composition can be achieved if the product ion process is conducted at its optimum conditions. Having a clear and complete understanding of the effect of the process variables on the product yield is, in fact, the key for process optimization.

The glycerolysis process can be carried out using mineral catalysts (1, 2) or biological catalyst (3-5). Examination of the previous work reported in the literature has shown that a great deal of work has been made on the glycerolysis process using mineral catalysts. This process, however, has so many disadvantages (6) so that recent research trends in the world is now to use biological catalysts; lipase enzyme, as a substitute of mineral catalysts (7-9). However, which lipase preparation is technically and economically most suitable to catalyze the process is still a unanswered question.

The aim of this work is to investigate the effect of the process variables on the yield of partial glycerides by indirect esterification of vegetable oils with glycerol in presence of lipase enzyme as catalyst.

2. EXPERIMENTAL

1. Materials

The materials used to prepare partial glycerides by glycerolysis were sunflower oil, glycerol and lipase enzyme as catalyst. Sunflower oil (refined,

bleached and deodorized) was kindly supplied by the Extracted Oils and Derivatives Company, Damanhour plant, Damanhour, Egypt. This oil was chosen as it is one of the main vegetable oils produced in Egypt. The major fatty acid of sunflower oil is linoleic acid (52 %) and the total unsaturation is about 83%.

The glycerol used was a redistilled brand (99 % pure) and was a product of El-Nasr Company for Pharmaceuticals, Egypt.

The lipase preparations used in the present work include the following:

a) *Pancreatic lipase* (crude steapsin from porcine pancreas);
a product of Sigma Chemical Company, USA. The protein concentration was 25 % and the lipolytic activity was 39 units/mg protein.

b) *Chromobacterium lipase* (from *Chromobacterium Viscosum*)
It is a product of Sigma Chemical Company, USA, in the form of a lyophilized powder containing approximately 65 % protein and its activity was 3000 units/mg protein. This lipase represents a bacterial lipase.

c) *Rhizopus Arrhizus Lipase*
It is a product of Sigma Chemical Company, USA, in the form of suspension in 3,2M $(NH_4)_2SO_4$, and 10 mM potassium phosphate solution (pH = 6). Its protein content was 12.7 mg protein/ml and its activity was 400,000 U/mg protein. This type is a fungal lipase.

d) *Lyophilized lipase (plant lipase) from wheat germ*
It is a product of Sigma Chemical Company, USA, in the form of a powder containing 95 % protein and its activity was 74 U/mg protein.

e) *Two lipase preparations* kindly supplied as a gift by Nagase Biochemicals LTD Company, Japan. They were derived from *Rhizopus japonicus* given the code *Lilipase A-10* and *Lilipase B-2* and their lipolytic activities were 1,000,000 and 100,000 U/g respectively.

2. Glycerolysis procedure

In stoppered conical glasses, 100 ml capacity, mixtures of 30 g glycerol, 10 g oil, 1.5 g water and lipase catalyst (equivalent to 7000 activity units) were incubated in a thermostatically temperature - controlled shaker. The temperature ranged from 30 to 45°C and the incubation period from few hours up to three days. After the incubation period, the reaction was stopped by adding 60 ml of chloroform and an equal amount of water to inactivate the enzyme. The lipid fraction containing the tri,di and mono-glycerides and fatty acids was extracted in the chloroform layer whereas the unreacted glycerol was separated in the water layer. In order to remove any residual traces of glycerol in the chloroform extract, the latter was washed three times with distilled water.

The chloroform extract was then shaked with anhydrous sodium sulfate to remove the water traces, filtered and the solvent was distilled-off at 60°C under vacuum. The extracted lipids were then cooled and stored at 5°C for further analysis.

3. Analytical Methods

The lipid constituents of the reaction products of the glycerolysis process were determined by thin layer chromatography (TLC).

Chromatoplates (20 X 20cm) were coated with a slurry of silica gel (60G) in water (30 g solid/100ml water), air dried and then activated at 110°C for one hour. The collected samples and standard samples of free fatty acids, mono-,di-, and triglycerides were then spotted on the activated thin layer plates, 3 cm from the bottom. The developing jar was prepared 15 minutes before insertion of the plates. The jar was lined on three sides with filter paper wetted with the same developing solvent. The plates were developed till the solvent front reaches 15 cm from the start line.

The plates were then air dried and sprayed lightly with chromic-sulfuric acid mixture (75 parts conc. H_2S0_4 + 25 parts saturated potassium dichromate). The sprayed plates were then charred in an oven adjusted at 180°C for 30 minutes. The cooled charred plates were then scanned on TLC Densitometer whereby the recorded peak area of each charred spot is proportional to the carbon density of that spot which, in turn, is proportional to the separated amount of each component on the plate. Percentage peak area of a certain component can then be taken as a measure for percentage weight of that component in the spotted sample.

3. RESULTS

The results of sunflower oil glycerolysis in presence of lipase enzyme as catalyst are graphically represented in Figs 1-4. In Fig 1, the six lipases used in the present study are compared for their effect on the conversion percent of the triglycerides due to glycerolysis. The incubation temperature was 40°C and the incubation period was 72 hrs which was sufficient period for the oil-glycerol system to attain its equilibrium state. It can be seen that the lipase activity to catalyze the glycerolysis process decreases in the following order: *Chromobacterium lipase* > *pancreatic lipase* >

Lilipase A-10 > Lilipase B-2 > Rhizopus lipase > Lyophilized lipase. The origin of the most active preparation is bacterial whereas that of the least active is plant (wheat germ).

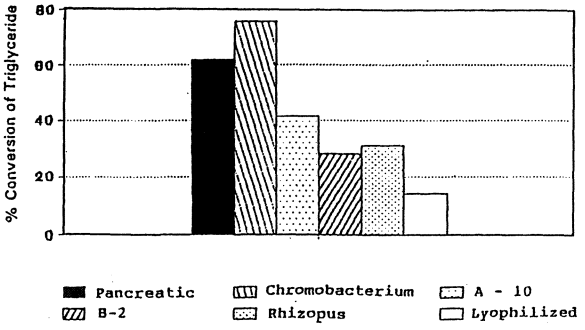

Figure 1
Percentage conversion of triglyceride after three days incubation at 40 °C for different enzyme preparations

The effect of the process temperature on the conversion rate of triglycerides, yield of partial glycerides obtained at equilibrium and the polarity of that equilibrium product was studied using the three lipase preparations which show promise as glycerolysis catalyst; Chromobacterium, pancreatic and Lilipase A-10.

It is clear from Fig 2 that the greatest yield of partial glycerides (62%) can be obtained using *Chromobacterium* lipase at 40°C which is the optimum process temperature using this preparation.

However, *Chromobacterium* lipase is considered one of the most expensive preparations (60.07 $/1000 activity units) (10). With pancreatic lipase, the process yield varies only slightly with temperature change in the range from 30 to 45°C, and it is almost the same at 40 and 45°C (52 %). Compared to *Chromobacterium* lipase, the pancreatic preparation is much cheaper (1 $/ 10^5 activity units) (10) and it can however be as efficient as *Chromobacterium* at 35°C and slightly more efficient at 45°C.

The lipase type and the process temperature affect, not only the product yield but also the ratio of monoglycerides to diglycerides in that product. The glycerolysis product usually contains beside mono-and diglycerides, some free acids (due to triglyceride hydrolysis) and residual unreacted triglycerides. These two latter components are usually removed during the purification steps by neutralization and fractional distillation. The purified product will be essentially a mixture of mono- and diglycerides and the product polarity will vary according to the proportions of these two glycerides.

If in a certain sample, the percentage moles of monoglycerides is M° and that of diglycerides is D°, then the ratio, α, between the total number of polar groups (OH) to non-polar groups (fatty acid radical) can be estimated from the relation:

$$\alpha = (2\,M^\circ + D^\circ) / (M^\circ + 2D^\circ).$$

In this study, it will be referred to this ratio α-as an index for the product polarity. M° and D° can be determined from the relation: M° = (M/356)(100/n) and D° = (D/620)(100/n). M and D are the weight percent of mono-and diglycerides, respectively and n is the total number of moles in 100 g sample. Total number of moles, n, can be estimated from the relation:

$$n = M/356 + D/620 + T/884 + F/282$$

In this equation T and F are the weight percentage of triglycerides and free fatty acids, respectively whereas the figures 356, 620, 884 and 282 are the average molecular weights of mono-, di- and triglycerides and fatty acids.

It should be pointed out that blends richer in monoglycerides which are more polar are suitable emulsifiers for certain food systems whereas those richer in diglycerides (less polar) are more suitable for other systems.

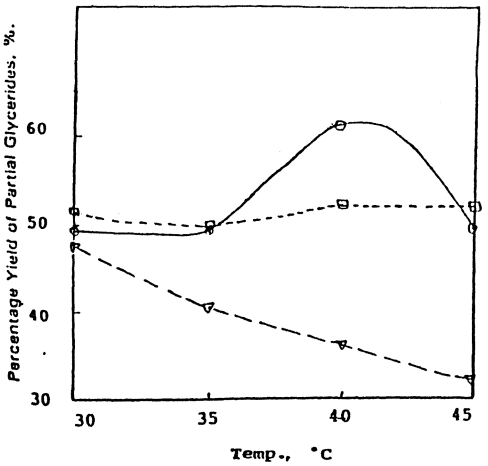

Figure 2
Percentage yield of partial glycerides at equilibrium at different temperatures for three enzyme preparations; (——) Chromobacterium, (- - -) Pancreatic and (— —) A-10.

The results represented in Fig 3 show the effect of enzyme type and process temperature on the polarity index, of the partial glycerides product obtained at equilibrium. It is obvious that the yield obtained using *Chromobacterium* lipase is less polar than that produced using pancreatic lipase or lilipase A-10 at 35 to 40°C. It can be also observed that the temperature effect on the product polarity using different lipases is quite variable. With pancreatic lipase, the product polarity varies very little due to

temperature changes whereas with *Chromobacterium,* more polar products can be obtained by processing at higher temperatures. The most polar products can be obtained using lilipase A-10 at 35°C. Processing at temperatures lower or higher than 35°C using this enzyme yields less polar products.

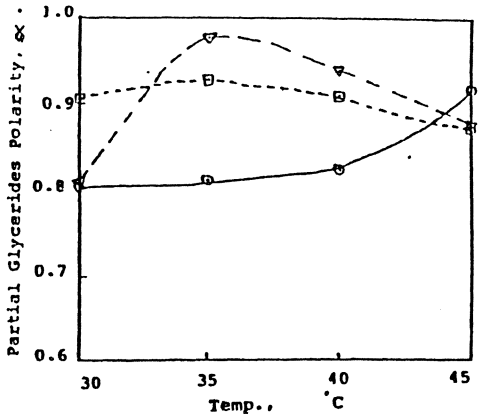

Figure 3
The effect of reaction temperature on the polarity index, α, for different enzyme preparations; (——) Chromobacterium, (- - -) Pancreatic and (— — —) A-10.

In fact, the yield and composition of the product at equilibrium are not the only important criteria of a sucessful glycerolysis process. Another important criterion is how fast the triglycerides conversion occurs. Fig. 4 shows the rate of triglyceride conversion at 40°C using the three lipase preparations. Conversion percent of the triglycerides in the system refers to the percentage amount of triglycerides consumed by conversion to mono-, diglycerides or free fatty acids.

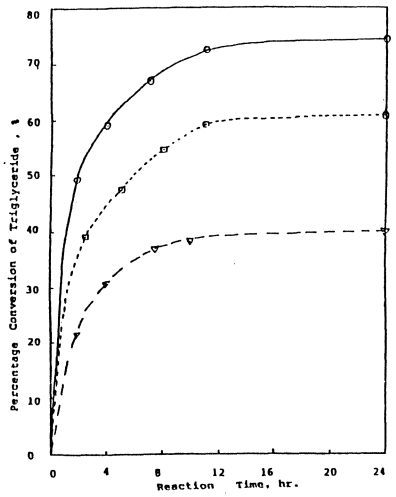

Figure 4
Conversion rate of triglyceride during oil glycerolysis at 40 °C using different enzyme preparations; (——) Chromobacterium, (- - -) Pancreatic and (— — —) A-10.

It is obvious that the lipase activity to accelerate the process of oil glycerolysis increases in the following order:

Lilipase A-10 < Pancreatic lipase < *Chromobacterium* lipase. The calculated initial conversion rates are 10.7, 15.64 and 24.4 % per hour using the three preparations, respectively in the same order.

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